



Responses of lake phytoplankton communities to changing inorganic nitrogen supply forms

Gabriele Trommer^{1,2} · Monika Poxleitner¹ · Herwig Stibor¹

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Abstract

A shift has been predicted in future nitrogen emission scenarios from nitrous oxide to higher proportions of ammonium compounds. To investigate the interaction between increasing nitrogen load and varying nitrate:ammonium ratios ($\text{NO}_3^-:\text{NH}_4^+$), we performed a mesocosm experiment in an oligotrophic lake in southern Germany. We fertilized mesocosms with both roughly natural and four times the natural nitrogen wet deposition amounts in molar $\text{NO}_3^-:\text{NH}_4^+$ ratios of 4:1 and 1:4. We observed greater phytoplankton biomass in treatments with a relatively higher ammonium supply, but not in those with nitrate and total nitrogen load. Ammonium significantly increased the total chlorophyll *a* concentrations, and especially the growth of small nanophytoplankton species. The effects observed indicate that NH_4^+ was taken up preferentially and that spring phytoplankton in oligotrophic lakes appear to be able to respond to variations in nitrogen forms (available $\text{NO}_3^-:\text{NH}_4^+$ ratios) by adjusting their community composition. Such communal changes at the base of the food web may affect higher trophic levels. Therefore, the effects of varying available forms of nitrogen should also be considered in primarily phosphorus-limited aquatic systems.

Keywords Phytoplankton · Community composition · Nitrate · Ammonium · Ratio · Oligotrophic

Introduction

Anthropogenic intervention in the global nitrogen (N) cycle is an issue of major interest, as increasing N loads often cause measurable changes in ecosystem functioning (Vitousek et al. 2010; Paerl et al. 2014; Steffen et al. 2015). In coastal ecosystems, the effects of N enrichment are usually immediately visible as typical signs of eutrophication, such as hypoxia and harmful algae blooms (Paerl 1997; Diaz and Rosenberg 2008; Paerl and Huisman 2008). Besides surface runoff and groundwater input, the greatest threat is the non-point, diffuse deposition of N compounds via the atmosphere, which can even reach remote areas (Bergström et al. 2005; Kim et al. 2014).

Reactive N compounds include nitrogen oxides (NO_x^-) and ammonium (NH_4^+), which have been increasing globally due to human activities over the last century (Galloway et al. 2003). A recent analysis revealed that NO_x^- production is currently more or less stable, but that NH_4^+ production continues to rise due to steadily rising human food requirements (Ciais et al. 2013). It is predicted that N fertilizer surpluses will continue to increase by a global average of 23% until 2050, and by up to 75% in Latin America (Bouwman et al. 2013). This may eventually double the recent average N deposition in industrialized and developing countries from 10 to 20 kg N ha⁻¹ year⁻¹ until 2050, and to over 50 kg N ha⁻¹ year⁻¹ in some Asian regions (Galloway et al. 2004). Therefore, future ecosystems face not only a continual accumulation of total N load, but also a shift in reactive N sources from NO_x^- towards reduced N forms.

In addition to the necessary management of total N load (and of course of total phosphorus) (e.g. Lewis et al. 2011; Paerl et al. 2016), it is recommended that, whenever possible, nitrogen management should focus on the control of bioavailable N forms (Lewis et al. 2011). The rapid recycling of inorganic and organic N complicates the management of specific bioavailable N forms. This is especially pertinent in

✉ Gabriele Trommer
gabi.trommer@gmx.de

¹ Department II Biology, Aquatic Ecology, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

² Present Address: Water Management Office Ansbach, Dürrenstr. 2, 91522 Ansbach, Germany

N-limited coastal waters, where NH_4^+ stimulates greater biomass development than nitrate (NO_3^-) when both are supplied in equal quantities (Paerl 1997). It is presumed that N is the main limiting macronutrient globally (Vitousek and Howarth 1991; LeBauer and Treseder 2008), hence the focus on both NO_3^- and NH_4^+ in N-limited systems. In terms of energy, the qualitative differences in NO_3^- and NH_4^+ supplies should influence primary production. In aquatic ecosystems, the primary producers can assimilate NH_4^+ directly, without need for costly enzyme activity to reduce NO_3^- (Eppley et al. 1969b; Falkowski 1975). Physiological adjustments of phytoplankton, such as suppressed nitrate reductase synthesis and activity, can occur when NH_4^+ concentrations exceed 0.5–1 μM (Eppley et al. 1969a). Various laboratory studies have observed differential utilization of N compounds by phytoplankton (Dortch 1990; Levasseur et al. 1993). A preference for NH_4^+ as the N source, for example, was noted in algal groups such as flagellates, green algae and cyanobacteria (Blomqvist et al. 1994; Dortch 1990; Domingues et al. 2011; Donald et al. 2013; Flores and Herrero 2005; Glibert et al. 2016), which have higher uptake affinities for this compound (Litchman et al. 2007). However, growth rates may be higher under NO_3^- supply, especially for a range of diatoms (Dortch 1990; Levasseur 1993; Litchman et al. 2007). In addition, size- or surface-to-volume ratios affect nutrient uptake, and smaller algae are known to be good competitors for nutrients such as phosphorus (P; Smith and Kalff 1982), as well as for NO_3^- and NH_4^+ (Eppley et al. 1969b; Hein et al. 1995; Wafar et al. 2004). On the other hand, there is evidence that larger algae species perform better with NO_3^- , and that they can also accumulate nutrients in vacuoles (Stolte and Riegman 1995). This was observed in diatoms and dinoflagellates, where maximum NO_3^- uptake rates per cell increased with cell size (Aksnes and Egge 1991; Litchman et al. 2007). Since the traits of phytoplankton groups differ with regard to nutrient use, a shift in N sources may alter the phytoplankton community composition through resource competition (Tilman 1977; Sommer 1985). Several field studies demonstrated that N forms are able to change the relative abundance of diatoms and cyanobacteria (Hutchins et al. 2003; McCarthy et al. 2009), and the relative contribution of chrysophytes and dinoflagellates to total phytoplankton biomass (Blomqvist et al. 1994; Poxleitner et al. 2016).

In the traditional context of ecological stoichiometry theory and nutrient limitation, attention has largely been focused on the general N:P supply ratio in ecosystems. The subject of N addition in N-excess systems has rarely been directly investigated (Poxleitner et al. 2016; Trommer et al. 2017; Lorenz et al. 2019). Lakes are naturally co-limited by N and P, or are seasonally limited by either of the two nutrients (Elser et al. 2007; Sterner et al. 2008; Paerl et al. 2016). On average, seston is characterized by a mean elemental ratio of N:P

between 16:1 (Redfield ratio) and 20:1 (Sterner et al. 2008). Therefore, in rather P-deficient systems at dissolved N:P ratios beyond 45:1 (Klausmeier et al. 2004), additional N was not initially expected to have a considerable effect on the natural phytoplankton. However, the nutrient identity of N, and not only supply rate, has been described to affect uptake and internal concentration of other nutrients such as P (Bracken et al. 2015). It has recently been shown that total N load and bioavailable N forms do matter and do indeed influence the primary production, including in rather P-limited systems (Lewis et al. 2011; Paerl et al. 2016). Furthermore, investigations have revealed that manipulating N availability in mesocosm studies in oligotrophic lakes resulted in changes in the phytoplankton community composition (Blomqvist et al. 1994; Poxleitner et al. 2016).

Although differences in the bioavailability of NO_3^- and NH_4^+ are indicated from laboratory studies, the effects of varying NO_3^- : NH_4^+ supply ratios in natural systems are considerably less known. Experiments on N resource use in natural communities have either been performed with a single N form (Axler and Reuter 1996) or in eutrophic, N-limited systems with combined N forms in constant fertilization ratios (Twomey et al. 2005; Donald et al. 2013). Furthermore, it is important to understand the potential interactions between the two nitrogen supply forms (Flores and Herrero 2005; Gruber and Galloway 2008). For example, the presence of NH_4^+ can inhibit NO_3^- uptake by a reported threshold ranging from > 0.1, 1, 4, to 15 μM (Dortch 1990; Dugdale et al. 2007). The degree of inhibition has thereby been demonstrated to be a function of the exposure history of algae to experimental nitrogen enrichments (Dortch and Conway 1984).

In order to investigate the impact of N enrichment on natural phytoplankton communities at two different NO_3^- : NH_4^+ supply ratios (based on realistic atmospheric N deposition scenarios), we performed a mesocosm experiment in an oligotrophic lake. As the lake studied is primarily P-limited in spring, we hypothesize that (1) N enrichment does not increase phytoplankton biomass. According to previous studies on enrichment with NH_4^+ or NO_3^- (Dortch 1990; Sommer 1993; Donald et al. 2013), (2) specific algal groups, such as small flagellated or green algae, should benefit from high NH_4^+ supply ratios. Lastly, (3) we expect a higher resource use efficiency for phytoplankton at high NH_4^+ supply rates, since NH_4^+ uptake is energetically less costly in comparison to NO_3^- .

Materials and methods

Study site and experimental design

In spring 2014 (March 11–May 28, 2014, 77 days) a mesocosm experiment was conducted in Lake Brunnsee, a kettle

lake in Upper Bavaria, Germany (47° 15' N, 12° 12' E). This relatively small lake (area: 0.058 km², max. depth: 19 m) can be characterized as oligotrophic, with a low total phosphorus (TP) concentration of <0.3 μM and a low chlorophyll *a* concentration of on average <2 μg L⁻¹. At the time of the study, the lake water had mean concentrations of ~316 μM NO₃⁻ and ~1.2 μM NH₄⁺ and reached high dissolved N:P molar ratios of ~1000:1 after winter mixing.

The experimental setup consisted of twelve enclosures made from white, opaque polyethylene foil (150 μm, Biofil Film GmbH, Germany). These were sealed as closed-bottom cylindrical bags, with a length of 3 m and a diameter of ~0.95 m (~2100 L). They were gently lifted from a depth of ~8 m, filling them with a subsample of the natural phytoplankton and zooplankton community, and then secured to anchored rafts. As the enclosures were open to the atmosphere, they were covered with a frame and transparent foil in order to prevent nutrient input through precipitation. Sensors recorded the temperature hourly, and were installed in each enclosure at a depth of 1.5 m. On day 23, the light attenuation in the enclosures was measured with a spherical light probe (LI 1000, Licor, Lincoln, Nebraska, USA).

A 2 × 2 factorial design was applied (four treatments, each replicated three times) in order to test the effects of increasing N supply and varying NO₃⁻:NH₄⁺ supply ratios (Table 1). The N addition was performed based on a weekly, natural deposition of approximately 2 mM N per enclosure surface (~0.72 m²). Rain samples on the lake (Trommer, unpublished data) and monitoring data from the Bavarian Environment Agency and the German Meteorological Survey were used to estimate the natural N wet deposition. The mean regional precipitation was ~25 L m⁻² per week for the years 2012 and 2013. The concentrations of NO₃⁻ and NH₄⁺ in the rain samples were approximately 0.05 mM respectively, which adds up to a hypothetical supply of ~1 mM NO₃⁻ and NH₄⁺ per week and per enclosure surface. As N deposition is predicted to increase two- to five-fold by 2050 (Galloway et al. 2004), we decided to

simulate a four-fold increase of N supply. The quantitative effects of N increase were tested using both natural (1 mM fertilization⁻¹, 2 mM week⁻¹) and four-fold (4 mM fertilization⁻¹, 8 mM week⁻¹) input (Table 1). In order to examine the effects of different NO₃⁻:NH₄⁺ supply ratios, we chose an NH₄⁺-excess supply ratio of 1:4 NO₃⁻:NH₄⁺ based on future projection figures (Ciais et al. 2013) and a NO₃⁻-excess supply ratio of 4:1 NO₃⁻:NH₄⁺ to simulate the original lake water. The 2 × 2 factorial design resulted in four treatments with two N supply quantities (1 and 4 mM) and two N supply ratios (4:1 and 1:4 NO₃⁻:NH₄⁺) of N (Table 1), with treatment 1 (1 × 4:1) being the closest approximation to natural conditions. The experimental design also provided three different supply levels of NO₃⁻ and NH₄⁺ (0.2, 0.8 and 3.2 mM fertilization⁻¹; Table 1).

The N addition was performed twice weekly (Mondays and Thursdays) over the entire experimental period in order to simulate semi-continuous fertilization. The fertilization solutions for each mesocosm were freshly prepared in the laboratory prior to fertilization, using sodium nitrate (NaNO₃) and ammonium chloride (NH₄Cl) as N sources. The stock solutions for the 4:1 and 1:4 NO₃⁻:NH₄⁺ treatments contained: (I) 0.32 mM NaNO₃ + 0.08 mM NH₄Cl and (II) 0.08 mM NaNO₃ + 0.32 mM NH₄Cl. Depending on the treatment, 2.5 or 10 mL of either stock solution I or II was placed into a labeled one-liter bottle, which was subsequently filled up to the mark with distilled water (N fertilization scheme, Table 1). The bottles of fertilization solution were subsequently brought to the lake and emptied into the respective enclosures. Immediately afterwards, a Secchi disc was lowered into each enclosure in order to distribute the N in the water column. To ensure that differences were present early in the experiment, an initial fertilization containing a two-week N amount (4 fertilizations) was performed on March 13, 2014. The experiment was conducted for a total of 11 weeks, and included 22 fertilizations.

Sampling program and laboratory analysis

Water samples were taken twice weekly with an integrated water sampler (KC Denmark A/S Research Equipment, Denmark) from a depth of 0.5–2.5 m. For all the following analyses, the water was pre-filtered directly on site using a 250-μm mesh in order to exclude mesozooplankton. The sampling time was kept as short as possible and the samples were stored in coolers for transportation. Within 1.5 h, the samples were in the laboratory's refrigerator (~8 °C), from where they were immediately processed. The chemical parameters of the water in each enclosure were measured weekly.

The nitrate and nitrite concentrations were determined by means of ion chromatography on particle-free water (Dionex ICS-1100 Basic Integrated IC System with Dionex IonPac

Table 1 Experimental design: N fertilization scheme (three mesocosm replicates per treatment) with N quantities (1 mmol fertilization⁻¹; 4 mmol fertilization⁻¹), N ratios (4:1 and 1:4 NO₃⁻:NH₄⁺) and amounts of NO₃⁻ and NH₄⁺ supplied in mmol and mg (twice weekly)

Treatment	Fertilization scheme	N addition [mmol] per fertilization		N addition [mg] per fertilization	
		NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺
	Quantity-ratio				
1	1 × 4:1	0.8	0.2	49.6	3.6
2	1 × 1:4	0.2	0.8	12.4	14.4
3	4 × 4:1	3.2	0.8	198	14.4
4	4 × 1:4	0.8	3.2	49.6	57.6

AS22; Thermo Scientific, USA; detection limit 2 μM). Prior to filtration in the laboratory, the syringe filters (0.45 μm , CS 400 Syringe Filters Cellulose Acetate Nalgene, USA) were rinsed several times with deionized water. Subsequently, 2-mL aliquots of enclosure water was filtered and ~ 1 mL of the particle-free water was injected into the Dionex (20 μL were sampled for the measurement).

Ammonium was determined using the orthophthalate method on the 250 μm pre-filtered water samples, which was modified from Holmes et al. (1999). Immediately after returning to the laboratory, the pre-filtered water samples were processed by mixing a 2.5-mL aliquot of enclosure water with 10 mL of working reagent (borate buffer, sodium sulfite and orthophthaldialdehyde in ethanol) in a scintillation vial. Following a 2-h dark incubation, the yellow-colored complex was measured in a Trilogy Laboratory Fluorometer (Module CDOM/NH₄; Turner Designs, USA).

Soluble reactive P (SRP) was not measured since previous studies had indicated that it was rarely measurable in Lake Brunsee, and we therefore focused on total P (TP). 12 mL of enclosure water was analyzed for TP, using the molybdenum blue reaction following digestion with 0.7 mL of sulfuric acid. Subsequently, 0.7 mL of ascorbic acid and 0.7 mL of a reagent mixture (sulfuric acid, antimony potassium tartrate and ammonium molybdate solution) were added. The blue color complex was measured with a spectrophotometer at 880 nm after 30 min (Shimadzu UV-1700, Shimadzu Cooperation, Germany) (Wetzel and Likens 1991).

The particulate organic carbon (POC), particulate nitrogen (PN) and particulate phosphorus (PP) were analyzed in order to determine seston stoichiometry. 200 to 250 mL of enclosure water was filtered on glass fiber filters once a week (GF/F, 0.7 μm , precombusted for 4 h at 500 $^{\circ}\text{C}$ and washed in 8% hydrochloric acid prior to use). For the POC measurements, the filters were incubated with phosphoric acid (0.5 M) for 30 s to evaporate the calcite. All the filters were directly frozen at -20 $^{\circ}\text{C}$ until the measurements. As with the TP value, the PP was analyzed using the molybdenum blue reaction after the filter was digested in 12 mL of distilled water and 0.7 mL of sulfuric acid (Wetzel and Likens 1991). The POC and PN filters were dried for 24 h at 60 $^{\circ}\text{C}$, placed in tin foil and measured with an elemental analyzer (vario MicroCube, Elementar, Germany).

To document phytoplankton development, the chlorophyll *a* concentrations were measured twice weekly in vivo using spectral fluorometry (AlgaeLabAnalyser, bbe Moldaenke GmbH, Germany). The water samples were kept in darkness until analysis (~ 1.5 h) in order to obtain the optimal fluorescence intensity (Maxwell and Johnson 2000). The AlgaeLabAnalyser measures chlorophyll *a* fluorescence at 685 nm following the excitation of the photosynthetic pigments by light-emitting diodes at five different wavelengths (450, 525, 570, 590 and 610 nm; for a detailed description,

see Beutler et al. 2002). In addition to total chlorophyll *a* concentration, it calculates the abundance of spectrally identified algal groups by fitting their pigment excitation spectra to so-called norm curves (Beutler et al. 2002). The four spectral groups identified are the brown (Heterokontophyta, Dinophyta), green (Chlorophyta), blue (Cyanobacteria) and mixed groups (Cryptophyta; for descriptions and validation of the method, see Beutler et al. 2002).

We followed the calculations of Ptacnik et al. (2008) to compare the resource use efficiency (RUE) of phytoplankton at different $\text{NO}_3^-:\text{NH}_4^+$ ratios. The RUE for each mesocosm was obtained by dividing the highest chlorophyll *a* concentration by the highest TP concentration (at the beginning of the experiment; $\text{RUE}_{\text{Chl } a} = \text{Chl } a/\text{TP}$).

To determine the phytoplankton biomass effects and the potential taxonomic shifts in the community, water samples were fixed in Lugol's iodine and the phytoplankton were later counted under an inverted microscope (Wild M40, Germany) following established methods (Uthermühl 1958). Samples from days 49 to 63 were analyzed, as the in vivo chlorophyll *a* measurements indicated treatment effects during this time period. To this end, 50 mL of the fixed water sample was placed in sedimentation chambers and, after settling for 48 h, 400 specimens of the most abundant species were counted in at least two rows. This was done at magnifications of 40 to 400, depending on algal size. The respective phytoplankton abundances were subsequently calculated as biovolumes by multiplying the cell counts by the species cell volume, which was either obtained through direct measurements (Hillebrand et al. 1999) or from existing data from previous studies on this lake.

The data were statistically analyzed using Systat Software 2008 (Sigma Plot 11.0). On the basis of the experimental design, two-way ANOVAs were performed with fixed factors (N amount and $\text{NO}_3^-:\text{NH}_4^+$ ratio) and the interaction term (N amount*ratio). The nutrient data were averaged over the experimental period. The averages for phytoplankton chlorophyll *a*, microscopic biovolume, seston stoichiometry, $\text{RUE}_{\text{Chl } a}$ and chlorophyll *a*: POC ratio were calculated over days 49 to 63 (maximal phytoplankton abundance). The maximum chlorophyll *a* concentrations were tested separately.

Results

Nitrogen dynamics

Significant differences in the amount of dissolved N compounds (NH_4^+ and NO_3^-) were established between the treatments (averages over time: NH_4^+ : interaction term $p < 0.001$; NO_3^- : interaction term $p < 0.001$). The temperature in the enclosures increased from ~ 7.4 (± 0.1 st.dev.) to

a maximum of $19.5 (\pm 0.1) ^\circ\text{C}$. The average light attenuation coefficient on day 23 was $0.83 (\pm 0.12)$, which was equivalent to a 10-m euphotic zone in the lake. No differences in the mean temperature and light attenuation coefficient were observed between the treatments on day 23 (averages over time: temperature: $p > 0.05$; light: $p > 0.05$ Kruskal–Wallis-test).

While the NH_4^+ concentration increased following the initial fertilization, it remained below $2.3 \mu\text{M}$ in treatment 1, and within a slightly unimodal range from 1.2 to $6.6 \mu\text{M}$ in treatments 2 and 3 (Fig. 1a); only treatment 4 obtained an increase of up to $28.3 \mu\text{M}$ (Fig. 1a). In the first 15 days after the start of the experiment, the NO_3^- concentration fell from $344 \mu\text{M}$ by $10 \mu\text{M}$ (treatment 3) to $24 \mu\text{M}$ (treatments 1, 2 and 4) (Fig. 1b). Following this period, it rose

to $358 \mu\text{M}$ in treatment 3, and hovered around $326 (\pm 3.4) \mu\text{M}$ in treatments 1, 2 and 4 (Fig. 1b). Meanwhile, the TP concentrations decreased from 0.27 to $\sim 0.05 \mu\text{M}$ and averaged around $0.16 \mu\text{M}$ (Fig. 1c), with no statistical differences noted between the treatments with regard to TP ($p > 0.05$).

Despite continuous N fertilization, the NO_3^- and NH_4^+ concentrations observed at the end of the experiment were below the theoretical endpoints (starting concentration plus all N additions, excluding any turnover) in all the enclosures (except for NO_3^- in No. 11; see Fig. 2a, b). From the amount of N missing from each mesocosm at the end of the experiment, we estimate a net N removal of $3\text{--}19 \mu\text{M}$ in NH_4^+ and -0.7 to $20 \mu\text{M}$ in NO_3^- (Fig. 2c, d), indicating mainly a phytoplankton uptake and a microbial turnover. The NH_4^+ removal increased with greater NH_4^+ addition

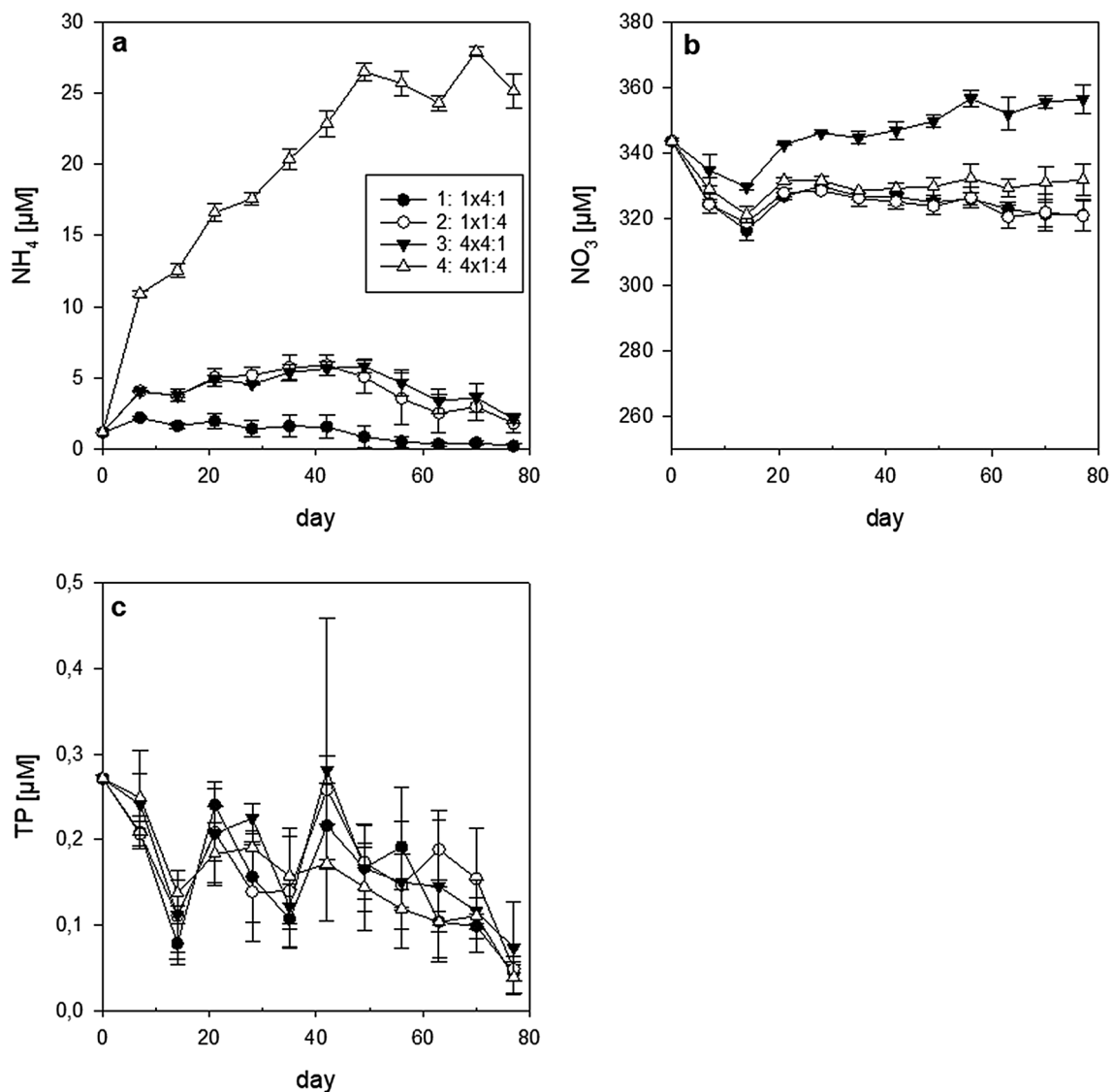


Fig. 1 Nutrient concentrations of **a** NH_4^+ , **b** NO_3^- and **c** TP (μM) are shown as averages of three replicates per treatment. The error bars represent standard deviation

(Fig. 2d, $R^2=0.95$, $p < 0.05$), but not with an increase in NO_3^- ($p > 0.05$). The removal of NO_3^- did not correlate with NO_3^- addition (Fig. 2c, $p > 0.05$), but it decreased with increasing NH_4^+ addition ($R^2=0.34$, $p < 0.05$).

Stoichiometric responses

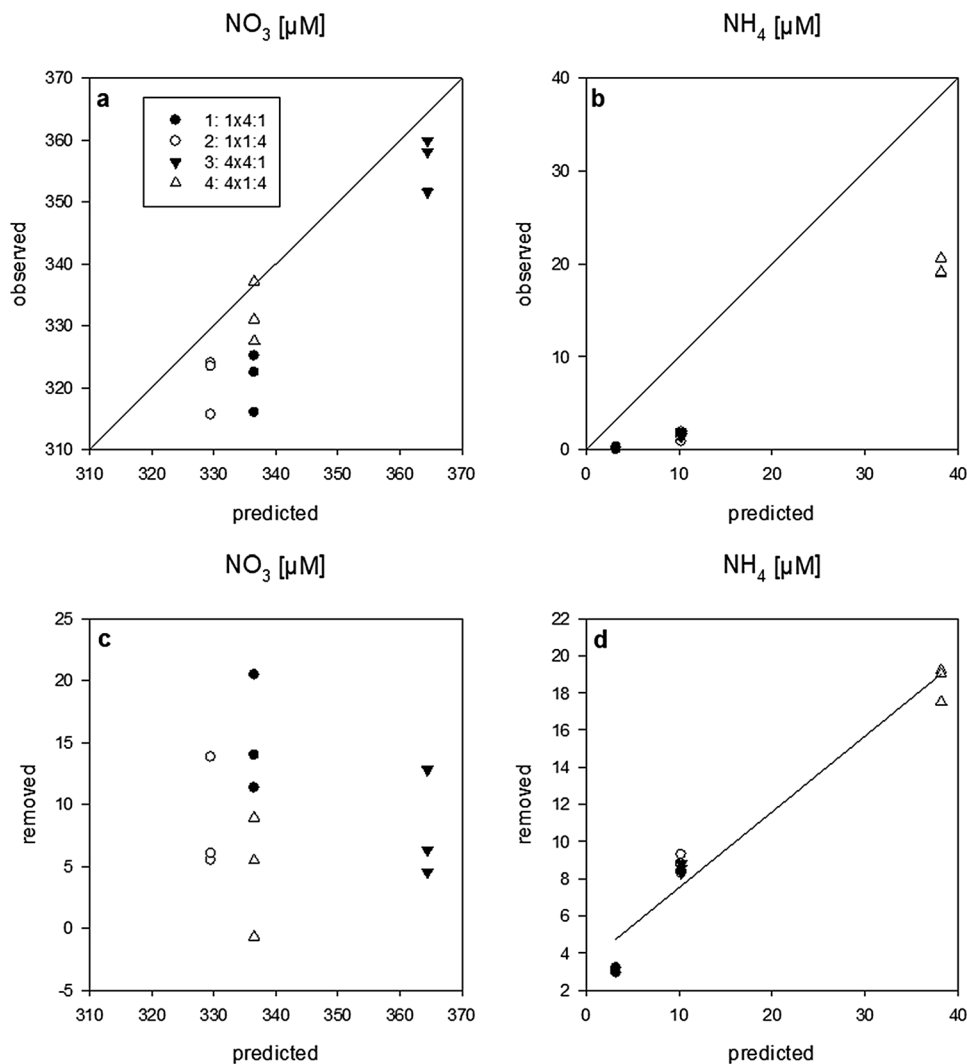
The seston stoichiometry data over the experimental period indicated P limitation. The seston C:P ratios increased from 252 at the beginning of the experiment to 634 (± 106) at the end, with an average of 498 (± 182). The seston N:P ratios averaged 83 (± 28), and rose from an initial value of 42 to a final value of 101 (± 23). The seston C:N ratios fluctuated between 3.1 and 9.1 (average 6.1 ± 1.3). No significant differences were observed for any of the stoichiometric ratios between the treatments over the chlorophyll *a* peak ($p > 0.05$). Lower seston C:N ratios were only observed in the fourfold N treatments on day 49 (N amount $p < 0.05$). On days 49 to 63, higher chlorophyll *a*-to-POC ratios were

observed in the NH_4^+ -excess treatment ($\text{NO}_3^-:\text{NH}_4^+$ ratio 1:4; N ratio $p < 0.05$), indicating more chlorophyll *a* production per unit particular organic carbon as a proxy for algae biomass.

Phytoplankton development and resource use efficiency

The total chlorophyll *a* concentrations reached maximum values of 2 to 6 $\mu\text{g L}^{-1}$ in early May, from days 49 to 59 of the experiment (Fig. 3a). During this period, the brown spectral group contributed $> 85\%$ of the total, followed by the green group (Fig. 3b, c). In the first month of the experiment, however, the mixed group also displayed a bloom, with a maximum chlorophyll *a* value of 1 $\mu\text{g L}^{-1}$ (Fig. 3d). By contrast, the blue spectral group only occasionally reached 0.01 to 0.04 $\mu\text{g L}^{-1}$ (not shown). Significant differences in total chlorophyll *a* were observed for the applied N ratio treatments at maximum chlorophyll *a* concentrations

Fig. 2 The observed versus the predicted end concentrations of **a** NO_3^- and **b** NH_4^+ (solid line equates $R^2=1$), and the removed versus the predicted end concentrations of **c** NO_3^- ($R^2=0.01$; $p > 0.05$) and **d** NH_4^+ ($y=0.41x+3.4$, $R^2=0.95$, $p < 0.001$). Predicted end concentrations are the theoretical endpoints of the starting concentrations plus all the N additions, excluding any turnover. The removed amount is the difference between the predicted and the observed end concentrations



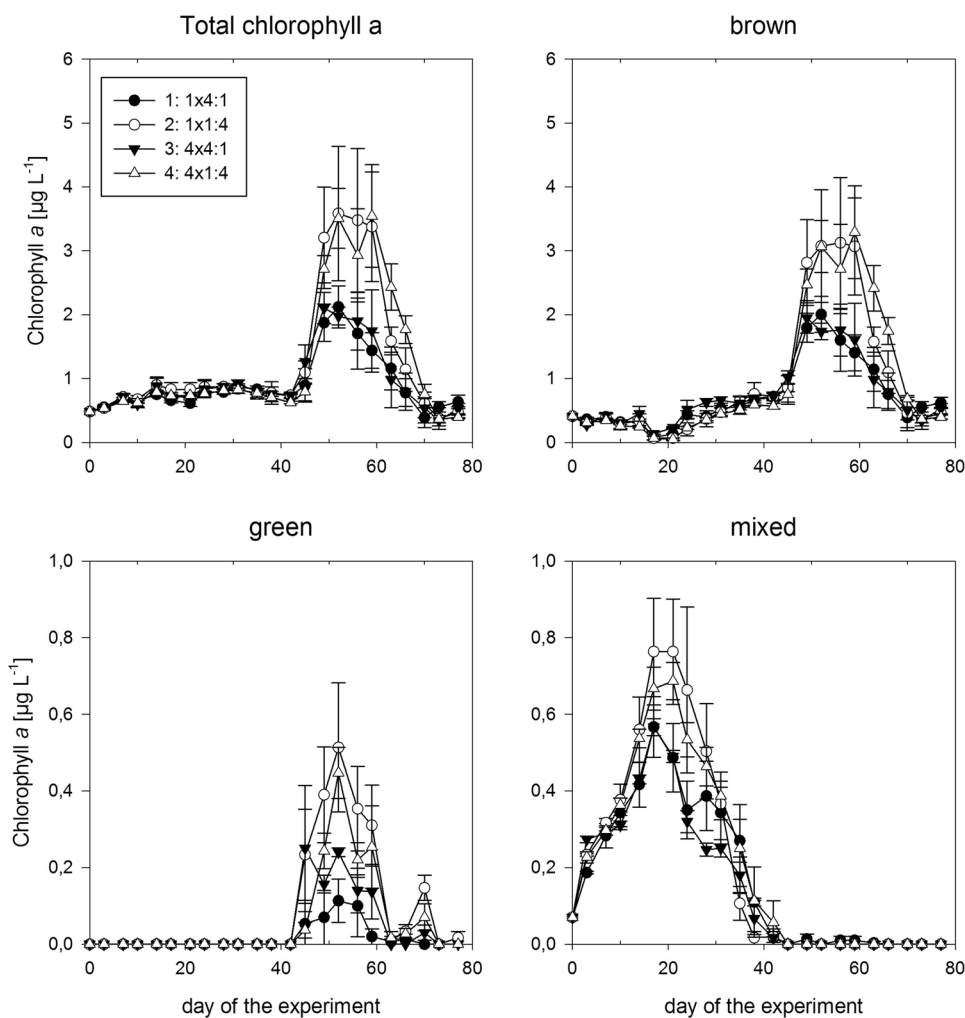
(Fig. 4, Table 2), as well as for the green and brown spectral groups (Table 2). In all the cases, the NH_4^+ -excess treatment ($\text{NO}_3^-:\text{NH}_4^+$ ratio 1:4) led to significantly higher chlorophyll *a* concentrations (Tukey Test N ratio $p < 0.05$, repeated measures ANOVA N ratio $p < 0.05$). No clear differences were noted between the N ratios for the mixed group during this time (Table 2), but they appeared earlier at their peak abundance on day 21 (N ratio $p < 0.05$). In addition, the $\text{RUE}_{\text{Chl}a}$ of the phytoplankton communities were significantly higher for both 1:4 $\text{NO}_3^-:\text{NH}_4^+$ treatments (N ratio $p < 0.01$) than they were for the 4:1 $\text{NO}_3^-:\text{NH}_4^+$ cases (Fig. 5), indicating greater biomass yield with a higher NH_4^+ supply.

Phytoplankton community composition

The phytoplankton biovolume calculated by means of microscopic counting was highest on days 49 and 59, reaching between 4.92×10^8 and $1.79 \times 10^9 \mu\text{m}^3 \text{L}^{-1}$ in the enclosures. We observed mean treatment differences for total phytoplankton biovolume on the basis of

the N ratio applied (Fig. 6 a, Table 3, Tukey Test N ratio $p < 0.05$, repeated measures ANOVA N amount and N ratio $p < 0.05$). These differences were mainly due to green algae, which proliferated in the NH_4^+ -excess treatments ($\text{NO}_3^-:\text{NH}_4^+$ ratio 1:4) (Fig. 6b). This was most prominently seen in the genus *Chlamydomonas* sp. (N ratio $p < 0.05$). Green algae comprised the largest proportion of total phytoplankton biovolume, with an average of ~67%, followed by diatoms (~29%) and other taxonomical groups (dinoflagellates ~2%, cryptophytes ~1%, cyanobacteria ~1% and chrysophytes <1%, Fig. 6b). The community was dominated by *Chlamydomonas* sp. (~65%) and *Cyclotella* sp. (~26%). *Ceratium hirudinella*, *Fragilaria* sp., *Asterionella* sp., *Crucigenia* sp. and *Rhodomonas* sp. accounted for only ~1 to 2% of the total biovolume, while *Dinobryon* sp. and *Anabaena* sp. made up <1%. No effects of the N fertilization applied were observed on the biovolumes of the diatoms, dinoflagellates, chrysophytes and cryptophytes (Table 3). The mean biovolume of the cyanobacteria during the peak phase was positively affected by the N amount supplied (Table 3).

Fig. 3 Chlorophyll *a* development over the experimental period: concentrations of total chlorophyll *a* and the chlorophyll *a* of the brown, green and mixed spectral algal groups. The averages of three replicates per treatment are shown. The error bars represent standard deviation



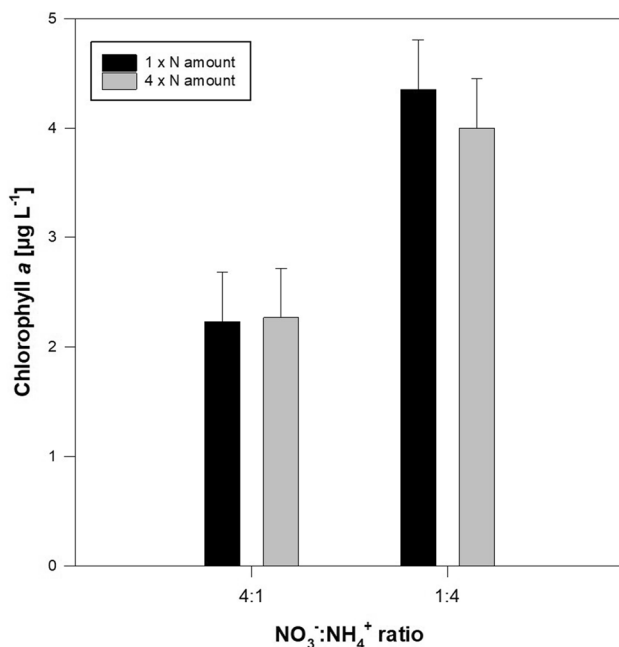


Fig. 4 Bar plot of maximum chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) versus $\text{NO}_3^-:\text{NH}_4^+$ treatments (4:1 and 1:4). The averages of three replicates per treatment are shown. The error bars represent standard deviation. Black bars (1): natural deposition, grey bars (4): four-fold natural deposition. $\text{NO}_3^-:\text{NH}_4^+$ ratio: $p < 0.01$

Table 2 Two way ANOVA results for chlorophyll *a*, with total N amount and $\text{NO}_3^-:\text{NH}_4^+$ supply ratios as fixed factors

	Total chl <i>a</i>	Green	Brown	Blue	Mixed
Amount	0.73	0.85	0.79	0.87	0.76
Ratio	0.003	0.02	0.004	0.43	<i>0.10</i>
Amount \times ratio	0.68	0.22	0.815	0.43	0.65

The *p*-values are shown for maximum concentrations of total chlorophyll *a*, and the respective concentrations of green, brown, blue and mixed spectral algal groups. Bold: $p < 0.05$, italic $0.05 < p \leq 0.1$

Discussion

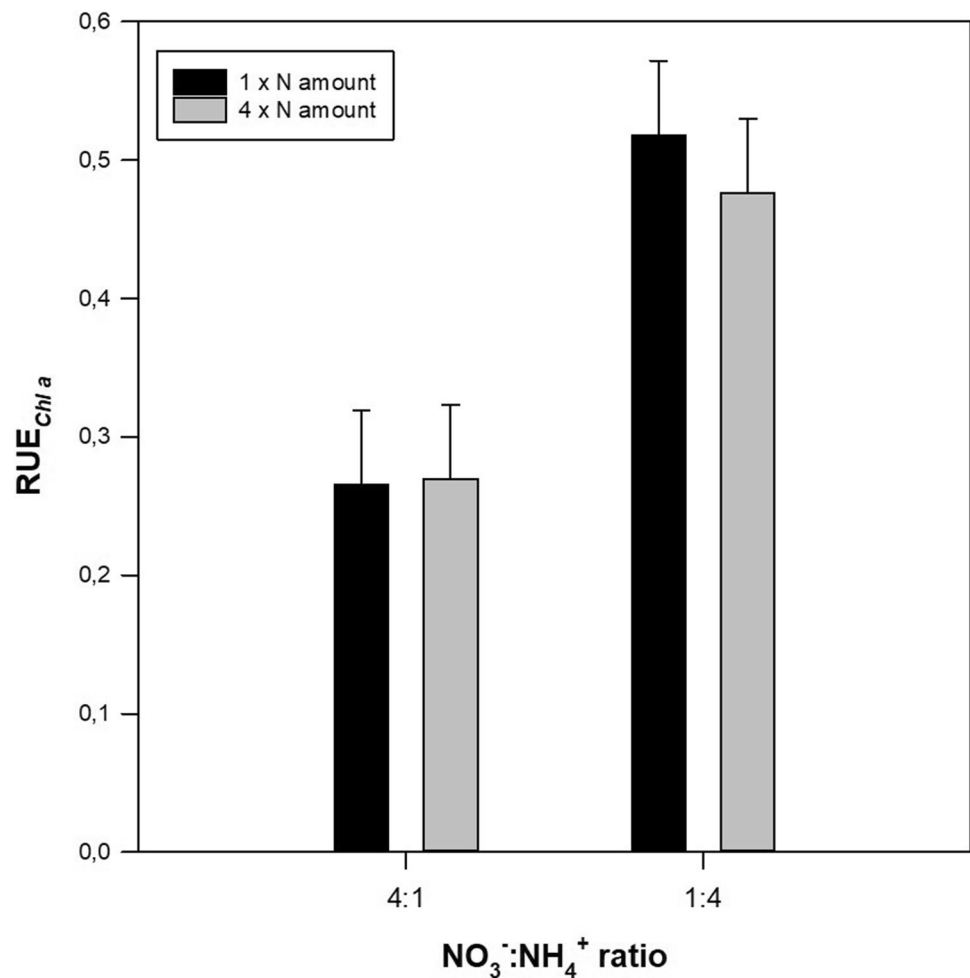
We investigated the effects of N enrichment on phytoplankton dynamics in a primarily P-limited, oligotrophic lake, using different N source ratios (NO_3^- , NH_4^+). The factorial experimental design applied had two N supply quantities and two $\text{NO}_3^-:\text{NH}_4^+$ supply ratios, and revealed clear effects of the supplied $\text{NO}_3^-:\text{NH}_4^+$ ratios on several aspects of the phytoplankton community. Until now, phytoplankton responses to NO_3^- and NH_4^+ have been mainly investigated in N-limited systems (Bergström and Jansson 2006; Duce et al. 2008; Donald et al. 2013). Lake Brunnsee, with TP $< 0.3 \mu\text{M}$ and dissolved molar N:P ratios of $> 1000:1$ (after winter mixing) indicating a severe scarcity of P, demonstrated a significantly higher

phytoplankton biomass in treatments with greater NH_4^+ fertilization (Tables 2 and 3, Fig. 4). This clearly suggests enhanced growth conditions for specific algal groups under higher NH_4^+ supply, which were evidenced by the chlorophyll *a* concentrations measured, the microscopically determined total phytoplankton biovolume and phytoplankton resource use efficiency, indicated by the amount of chlorophyll *a* per TP unit (Ptacnik et al. 2008).

With respect to the first hypothesis of this study, the N amount supplied did not affect the total phytoplankton biomass (Tables 2 and 3). The maximum phytoplankton biomass was achieved in treatments with 1:4 $\text{NO}_3^-:\text{NH}_4^+$ supply ratios. If absolute NH_4^+ availability had limited phytoplankton production in the lake investigated, the greatest phytoplankton biomass development would have been expected with the highest NH_4^+ supply (4 \times 1:4 $\text{NO}_3^-:\text{NH}_4^+$ ratio treatment, N fertilization per week: 6.4 mM enclosure⁻¹ or 2.9 μM), which was not the case. This observation might potentially be due to the growth inhibition of phytoplankton under high NH_4^+ conditions, which was already observed at NH_4^+ concentrations of about 10–100 μM (Yoshiyama and Sharp 2006; Dugdale et al. 2012; Glibert et al. 2016). In addition, a saturation effect related to the amount of available P could have limited the maximum biomass production at the highest NH_4^+ levels (Yoshiyama and Sharp 2006). At the given TP of $< 0.3 \mu\text{M}$, chlorophyll *a* concentrations of 4–7 $\mu\text{g L}^{-1}$ appear to be the upper range of attainable phytoplankton biomass (Fig. 2B in Ptacnik et al. 2008). In terms of N budgets, the absolute amounts of N removed from the dissolved phase were of similar magnitude for NH_4^+ and NO_3^- (Fig. 2c, d). The same NH_4^+ amount was added for the 1 \times 1:4 and 4 \times 4:1 $\text{NO}_3^-:\text{NH}_4^+$ ratio treatments (Table 1, Fig. 1a) and approximately the same amount was removed (Fig. 2d). Nevertheless, the greater biomass was observed in the elevated NH_4^+ treatments ($\text{NO}_3^-:\text{NH}_4^+$ ratio 1:4). These results clearly suggest NH_4^+ as the preferred growth- and biomass-promoting N source for the algae community in our experiment. Similar effects of significantly higher phytoplankton biomass with NH_4^+ rather than NO_3^- fertilization have been noted for cyanobacteria and chlorophytes (McCarthy et al. 2009; Domingues et al. 2011; Donald et al. 2011, 2013; Glibert et al. 2016 and references therein).

We observed that NH_4^+ removal from the dissolved phase increased with greater NH_4^+ supply (Fig. 2), although the reported nutrient concentrations may not fully reflect the in situ concentrations due to the continued activity of microbial processes within the sample bottle between water sample collection and processing. This removal includes not only algal N uptake, but also potential uptake and conversion by bacteria and abiotic transformations. The control measurements showed that the mesocosms were oxygenated over the entire depth ($\sim 10.5 \pm 2.5 \text{ mg L}^{-1}$) and that the pH was slightly alkaline (third week of the experiment $\sim 7.2 \pm 0.1$,

Fig. 5 Bar plot of the total $RUE_{Chl\ a}$ at maximum chlorophyll a concentrations versus $NO_3^-:NH_4^+$ treatments (4:1 and 1:4). The averages of three replicates per treatment are shown. The error bars represent standard deviation. Black bars (1): natural deposition, grey bars (4): four-fold natural deposition. $NO_3^-:NH_4^+$ ratio: $p < 0.01$



last week of the experiment $\sim 8.1 \pm 0.1$). Under these circumstances, considerable effects of NH_3 volatilization or denitrification were not expected. Phytoplankton are known to prefer the energetically favorable NH_4^+ . Therefore, a high NH_4^+ supply may be beneficial to certain phytoplankton taxa, but it can also inhibit NO_3^- uptake for other taxa, such as large diatoms, which prefer to use the latter compound (Eppley et al. 1969b; Sommer 1993; Glibert et al. 2016). Laboratory studies have revealed that NO_3^- uptake by algae can be efficiently suppressed with NH_4^+ (Eppley et al. 1969a; Cochlan et al. 1991; Hii et al. 2011) through reducing nitrate reductase activity (Lomas 2004; Song and Ward 2004). The inhibition of NO_3^- uptake reportedly starts at critical NH_4^+ concentrations of 1 to 15 μM , depending on the algal species and the total N levels (Dortch 1990; Glibert et al. 2016). In our experiment, the highest concentrations of $> 10 \mu M$ NH_4^+ occurred in treatment 4 ($4 \times 1:4$), and we can only speculate about the relative importance of NH_4^+ inhibition for NO_3^- uptake. However, whereas our data do not indicate significant differences in NO_3^- uptake between the fertilization treatments, it appears that NH_4^+ uptake increased with NH_4^+ addition, resulting in a higher phytoplankton biomass.

We therefore assume that the preferential uptake of NH_4^+ , rather than inhibiting the NO_3^- uptake by NH_4^+ , affected the N dynamics in our experiment.

The results of the microscopic analyses indicate that it was specifically smaller algae, such as *Chlamydomonas sp.*, that were positively affected by increased NH_4^+ availability, which supports our second hypothesis. This hypothesis is further supported by the chlorophyll a data of the mixed spectral algae group (Fig. 3d), which represent mainly small cryptophytes (Beutler et al. 2002). Small algae have high surface area-to-volume ratios, resulting in greater specific uptake and growth rates when nutrients are scarce. This was demonstrated by the uptake of P (Smith and Kalff 1982), NO_3^- and NH_4^+ (Eppley et al. 1969b; Dortch 1990; Hein et al. 1995). A marine study has indicated that picoplankton prefer NH_4^+ over NO_3^- , whereas larger net plankton (15–200 μm) display no preference for either source (Wafar et al. 2004). Our results support the findings that smaller algae appear to be more competitive under high dissolved N:P ratios than larger ones (Edwards et al. 2011). Moreover, they align with predictions that a shift to a higher availability of NH_4^+ in comparison to NO_3^- may favor small species

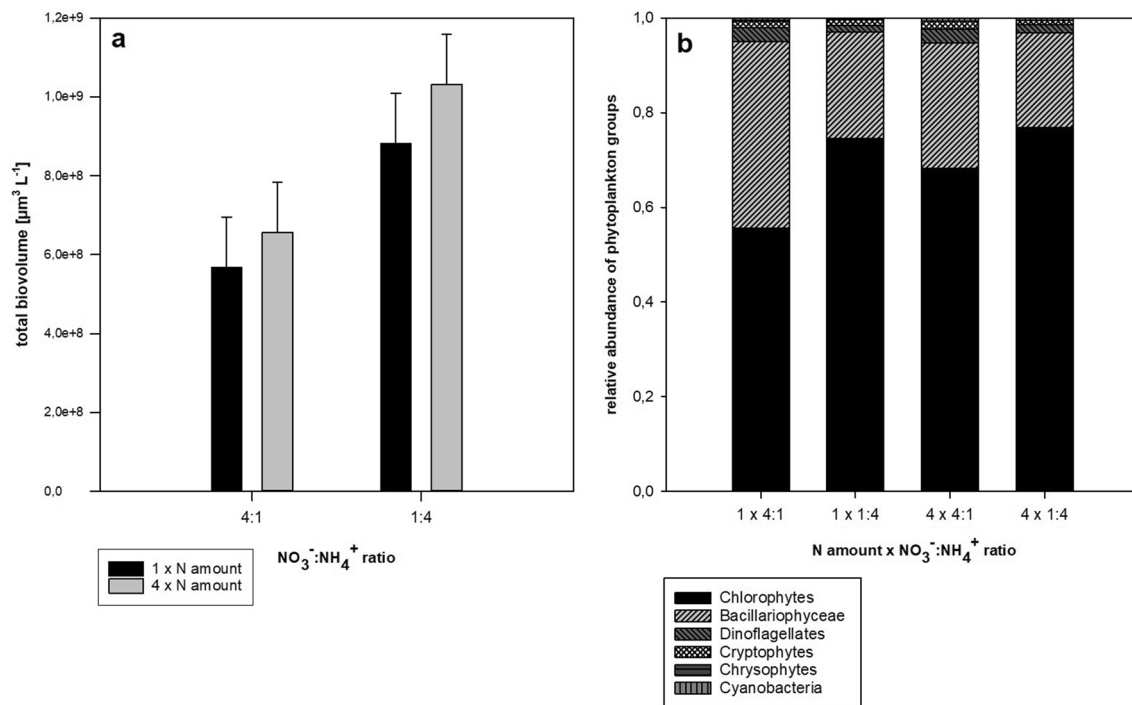


Fig. 6 **a** Grouped bar chart of average total phytoplankton biovolume [$\mu\text{m}^3 \text{L}^{-1}$] (days 49–63) versus $\text{NO}_3^-:\text{NH}_4^+$ treatments (4:1 and 1:4). The averages of three replicates per treatment are shown. The error bars represent standard deviation. Black bars (1): natural deposition,

grey bars (4): four-fold natural deposition. $\text{NO}_3^-:\text{NH}_4^+$ ratio: $p < 0.05$ and **b** stacked bar chart of the relative abundance of the phytoplankton groups versus the N treatments ($\text{N amount} \times \text{NO}_3^-:\text{NH}_4^+$ ratio)

Table 3 Two way ANOVA results for microscopic biovolume, with total N amount and $\text{NO}_3^-:\text{NH}_4^+$ supply ratios as fixed factors

	Total biovolume	Bacillario- phyceae	Chrysophytes	Dinoflagellates	Chlorophytes	Cryptophytes	Cyanobacteria
Amount	0.38	0.75	0.32	0.31	0.30	0.71	0.01
Ratio	0.03	0.95	0.61	0.40	0.02	0.57	0.86
Amount × ratio	0.82	0.66	0.16	0.84	1.00	0.50	0.37

The p -values are shown for the phytoplankton biovolumes, averaged over the chlorophyll a peak phase (days 49–63). Bold: $p < 0.05$

(Glibert et al. 2016). Their higher nutrient uptake rates and growth under low P (Smith and Kalff 1982) and NH_4^+ concentrations (Hein et al. 1995) mean that even slight NH_4^+ additions appear to be sufficient to promote the growth of small algae, which was observed in our field experiment. Our results indicate that the energetics of N assimilation lead to a preference for NH_4^+ as a nitrogen source, regardless of any nutrient deficiency (Syrett 1981; Dortch 1990).

The examination of the phytoplankton community composition revealed greater proportions of green algae with higher NH_4^+ availability ($\text{NO}_3^-:\text{NH}_4^+$ ratio 1:4; Tables 2 and 3). The most abundant algae in our experiment was the green algae flagellate *Chlamydomonas sp.* Averaging 67% of the algae biomass, its response to N enrichment (higher biovolumes in the NH_4^+ -excess treatments) was most probably

also the main driver of the response we observed (Table 3). These data agree with earlier findings that green algae—especially flagellated species—have higher uptake affinities and growth rates with NH_4^+ as a source (Dortch 1990; Raven et al. 1992; Litchman et al. 2007), and therefore profit from a high supply thereof (Domingues et al. 2011). Sommer's (1993) seasonal study of Lake Plußsee also noted the clear effects of NH_4^+ on phytoplankton species in a P-limited system. In addition, he observed a positive correlation between the abundance of three green algae species and decreasing $\text{NO}_3^-:\text{NH}_4^+$ ratios, as did our experiment. However, it is difficult to clearly link the recorded changes to a single factor in observational field studies. Therefore, our manipulation of NH_4^+ and NO_3^- supply ratios, while keeping other conditions constant, demonstrates a more causal relationship

between varying $\text{NO}_3^-:\text{NH}_4^+$ ratios and the observed responses of phytoplankton species.

Our data also support the third hypothesis that high NH_4^+ supply ratios increase the RUE of phytoplankton. Notably, the phytoplankton RUE was higher when relatively more NH_4^+ was supplied (under otherwise identical resource conditions; see Fig. 5). This result strongly suggests that the ratio between two available N sources can affect the transformation efficiency of available P into new biomass, and that the limiting nutrient P could be used more readily with NH_4^+ as the complementary N source. A previous study of optimal macro- and micronutrient conditions for several algal species observed a similar pattern (Radzun et al. 2015), with NH_4^+ resulting in higher growth than comparable NO_3^- concentrations for most of the species. In addition, P was converted more efficiently into new biomass in the presence of NH_4^+ instead of NO_3^- .

Conclusion

Our data suggest that nitrogen enrichment also affects phytoplankton dynamics in lakes with a presumed P scarcity. The main driver of the dynamics observed was NH_4^+ . With regard to future deposition scenarios, a restriction of N production and emission amounts could potentially alter the lake dynamics if the deposited N form shifts towards a relatively higher NH_4^+ deposition. Higher NH_4^+ ratios may increase the chlorophyll *a* concentrations and affect the growth of certain phytoplankton groups, together with the phytoplankton resource use efficiency. Such a change might also promote shifts towards higher proportions of small green algae, especially flagellated species (such as *Chlamydomonas sp.* in our experiment). A shift towards the more efficient utilization of P, combined with a shift towards highly-edible algae such as *Chlamydomonas sp.*, could result in greater zooplankton production and thus greater planktivorous fish production in P-limited lakes as NH_4^+ input increases. Changes in the supply ratio of N sources can therefore alter aquatic food webs, even in ecosystems where the element is not a limiting factor per se. Consequently, N management, as part of conservation strategies to ensure sustainable ecosystem functioning and services, should include not only N-limited ecosystems, but also largely P-limited freshwater lakes (Harpole et al. 2011; Lewis et al. 2011; Paerl et al. 2011, 2016).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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